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Histopathology of Canine Sporotrichosis: A Morphological Study of 86 Cases from Rio de Janeiro (2001–2007)

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Abstract The present study reports the histopathological findings of 86 skin lesions of dogs with sporotrichosis from Rio de Janeiro. Suppurative granulomatous inflammation was the predominant finding and was observed in 76 (88.37%) cases. Plasma cells surrounding the suppurative granulomas were detected in 68 (89.5%) cases and an inflammatory infiltrate at the periphery of these granulomatous lesions was observed in 63 (82.9%). Fungus-specific staining revealed yeast cells compatible with *Sporothrix schenckii* in 36 cases. These fungal elements were only detected in lesions characterized by suppurative granulomatous inflammation. Thus, specific staining of serial sections is recommended in the case of dogs with skin lesions whose histopathological presentation is consistent with sporotrichosis. However, due to the generally small number of yeast cells in lesions, the hypothesis of sporotrichosis should not be ruled out even if the result is negative, especially in epidemic areas where correlation with epidemiological data is particularly useful.

Keywords Histopathology · Sporotrichosis · Dog · Skin · Diagnosis

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Introduction

Sporotrichosis is a mycosis caused by the fungus *Sporothrix schenckii* [1]. Zoonotic transmission is characterized by skin inoculation of the agent through the bite or scratch of infected animals or by contact with the exudate of their lesions [2–5]. Domestic cats are the main source of zoonotic transmission of sporotrichosis because their lesions are rich in fungi [6].

Canine sporotrichosis is considered to be a rare disease, with only isolated cases being reported in the literature [7–9]. However, the Laboratório de Pesquisa Clínica em Dermatozoonoses em Animais Domésticos (LAPCLIN-DERMZOO), Instituto de Pesquisa Clínica Evandro Chagas (IPEC), Fundação Oswaldo Cruz (FIOCRUZ), has observed an increase in the number of cases of canine disease in Rio de Janeiro since 1998, a finding that seems to follow the epidemic described in humans which is related to feline transmission [4–6, 10, 11].

The definitive diagnosis of sporotrichosis is made by isolation and identification of the agent in culture [12]. However, this test may not be requested or available, and even when performed no fungal

growth may be observed, generally because of inadequate material or contamination with saprophytic microorganisms [13, 14]. In addition, the fungus can take weeks to grow, requiring maintenance of the culture for a long period of time [14].

Histopathological examination is an important tool for the diagnosis of sporotrichosis [15, 16]. In human sporotrichosis, skin lesions contain suppurative granulomas of the sporotrichotic type which are characterized by the presence of a suppurative central zone, a middle zone of mononuclear phagocytes and an outer zone of lymphocytes and plasma cells [13, 17].

Experimental studies have shown that the histopathological presentation varies according to the stage of the disease. First, the formation of abscesses and the presence of macrophages, lymphocytes and a large amount of fungi are observed. Once macrophages are activated and differentiate, forming granulomas, a decline in the number of fungi occurs which is accompanied by a decrease of abscesses and an increase in the number of plasma cells [18–20]. According to Adams [21], the sequential features of developing granulomas include morphological changes in mononuclear phagocytes. The mature macrophage in granulomas is a large polygonal cell with a large eccentric oval nucleus associated with slight blurring of cytoplasmic borders. In a later stage, granulomas mainly consist of epithelioid cells. These cells are large and elongated and present eccentric reniform nuclei and abundant pale eosinophilic and granular cytoplasm with indistinguishable borders.

The *S. schenckii* yeast cells are oval or cigar shaped, range in size from 4 to 6 μm [3], and generally exhibit single buds with a narrow base [14]. Histochemical staining techniques such as Grocott's silver stain and periodic acid Schiff (PAS) are useful to visualize yeast cells [12]. Nevertheless, identification of the etiological agent by morphological methods is not always possible because of the scarcity of *S. schenckii* yeast cells in human and canine sporotrichosis lesions [11, 16, 22, 23]. Immunohistochemical and molecular methods can be useful to improve diagnostic performance [24–29] and should be used when available.

Sporothrix schenckii asteroid bodies have also been described in tissue. These elements are identified by hematoxylin-eosin (HE) staining and are characterized by eosinophilic prolongations irradiating from a central yeast cell [13, 22, 30, 31]. Although this finding is frequently observed in sporotrichosis lesions, it is not specific of the disease [32, 33].

The microscopic aspects of skin lesions have been well described in both human and experimental sporotrichosis, but little is known about the disease in dogs. Studies regarding the histopathological alterations that occur in canine sporotrichosis are scarce and generally include reports of isolated cases [34, 35] and/or a short clinical and histopathological description of the lesions [11, 23]. In view of the current epidemic situation of sporotrichosis in Rio de Janeiro, the aim of the present study was to perform a systematic morphological analysis of canine sporotrichosis cases notified at LAPCLIN-DERMZOO, IPEC, since 2001, in an attempt to establish a characteristic histopathological profile of canine sporotrichosis in order to optimize the use of this test for the diagnosis of the disease.

Materials and methods

Sample

Eighty-six tissue samples of active skin lesions, from which *S. schenckii* was isolated by culture, obtained from dogs seen at LAPCLIN-DERMZOO, IPEC, FIOCRUZ, Brazil, between 2001 and 2007, were studied. Material obtained from dogs seen between 2001 and 2004 had been previously used by Schubach et al. [11] and Santos et al. [23] as part of a study on the general aspects of canine sporotrichosis.

Histopathology

The tissue samples embedded in paraffin blocks were recovered from the Serviço de Anatomia Patológica of IPEC and cut with a microtome. The sections obtained were stained with HE, PAS and Grocott's silver stain.

The type of inflammatory process—granulomatous or nonspecific—was determined by microscopic analysis of HE-stained sections. The cell types detected in the infiltrate were described and their presence was quantified as discrete to moderate or elevated.

Inflammatory infiltrates containing activated mononuclear phagocytes forming compact aggregates or abundant interstitial cords were classified as granulomatous. The predominant phagocyte differentiation

[21]—macrophages or epithelioid cells—was evaluated in these infiltrates. The granulomas were classified as well organized when they presented well-defined limits and a predominance of epithelioid cells, and as poorly organized when they were poorly delimited and/or presented a predominance of macrophages. The distribution of the granulomatous infiltrate was classified as diffuse, nodular or interstitial.

Inflammatory infiltrates without granulomatous features were defined as nonspecific inflammation. The distribution of non-granulomatous infiltrates was classified as diffuse, perivascular, perifollicular, interstitial or lichenoid according to Ackerman et al. [36].

Grocott's silver stain and PAS were used for the detection of fungal elements. For this purpose, all microscopic fields in two histological sections were analyzed per staining technique. Detection was considered to be positive when structures with consistent size and shape were observed.

Statistical Analysis

All data were stored and processed using the Statistical Package for Social Sciences (SPSS), version 11.0. The chi-square test of independence was used to determine a significant association between the variables studied. Fisher's exact test was applied to the comparison of variables with only two categories. A P value < 0.05 was considered to indicate a significant association.

Results

Microscopic analysis of the 86 lesions showed suppurative granulomatous inflammation in 76 (88.4%) cases, non-suppurative granulomatous inflammation in 1 (1.2%), and nonspecific inflammation in 5 (5.8%). No relevant histological findings were observed in 4 (4.6%) cases.

Suppurative Granulomatous Inflammation

The distribution of the granulomatous infiltrate was diffuse in 71.0% of the cases, nodular in 23.7% and interstitial in the remaining cases (5.3%). A predominant epithelioid differentiation of mononuclear phagocytes in granulomas was observed in 75.0% of the cases and differentiation into macrophages in 25.0%. Multinucleated giant cells were detected in only one case.

Well-organized granulomas (Fig. 1) were observed in 38.2% of the cases. Plasma cells were detected around granulomas in 89.5% of all cases ($n = 76$) (Fig. 2) and in 92.9% of lesions with epithelioid cell granulomas ($n = 57$). Poorly organized granulomas (Fig. 3) were observed in all cases in which this plasma-cell infiltrate was absent ($n = 8$) and in 57.3% of cases in which it was present ($n = 68$) ($P = 0.021$).

The number of neutrophils inside granulomas (Fig. 2) was high in 51.3% of the cases. The quantity

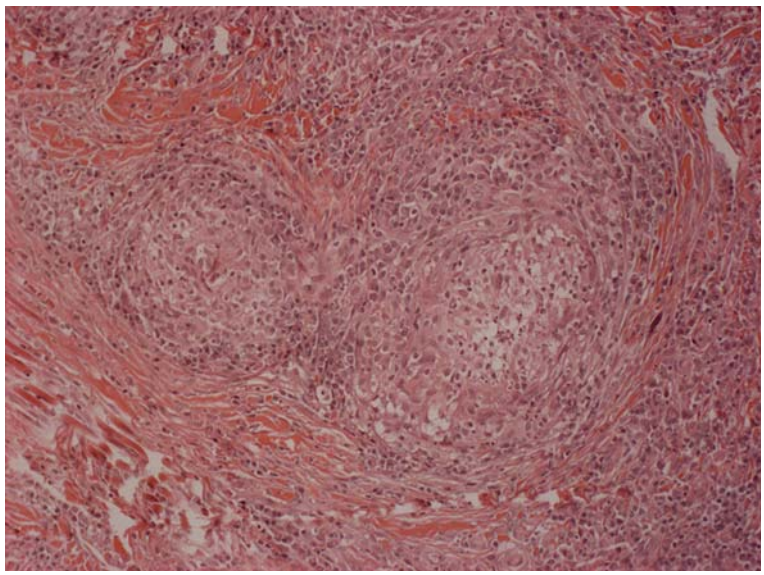

Fig. 1 Well organized granulomas. HE stain. 20×

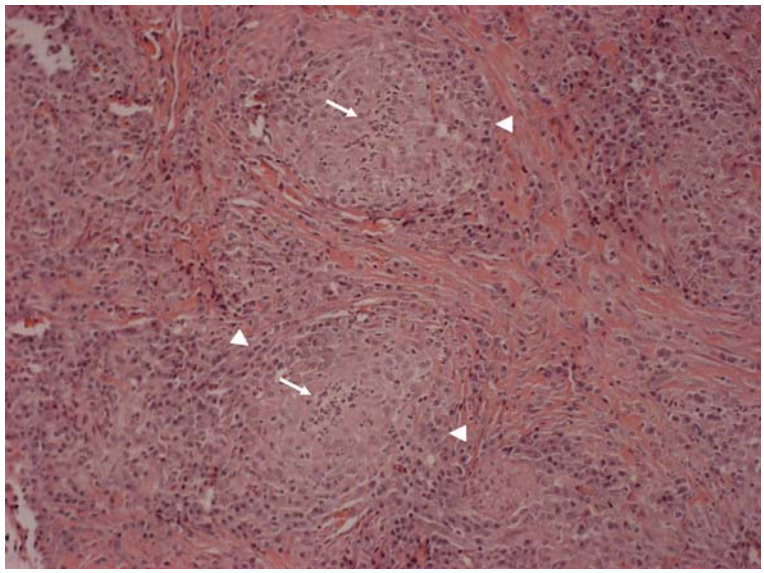

Fig. 2 Granuloma presenting neutrophils inside (*arrows*) and an outer zone of plasma cells (*arrow heads*). HE stain. 20×

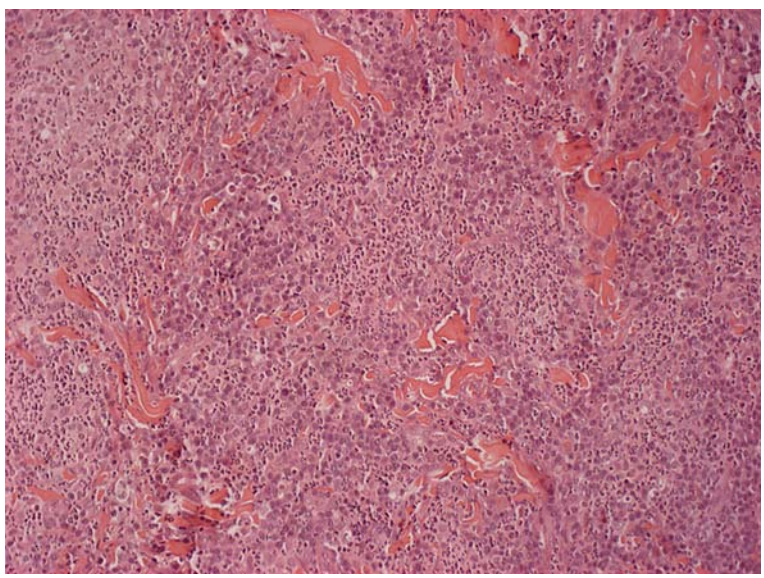

Fig. 3 Poorly delimited granuloma with predominance of macrophages. HE stain. 20×

of each cell type present in the infiltrate and its association with granuloma differentiation are illustrated in Table 1.

A non-granulomatous infiltrate at the periphery of the granulomas was observed in 82.9% of the cases. Plasma cells, detected in 81.6% of the cases, were the predominant cell type (Fig. 4). The distribution of the inflammatory infiltrate was perivascular, perifollicular and interstitial in 55 (72.4%) cases, with an associated lichenoid infiltrate being observed in 3. The distribution of the infiltrate was diffuse in 8 (10.5%) cases.

Neutrophils were detected in the non-granulomatous peripheral infiltrate in 68.4% of the lesions in which granulomas mainly consisted of macrophages ($n = 19$) and in 36.8% of those mainly consisting of epithelioid cells ($n = 57$), with this difference being significant ($P = 0.031$). The formation of microabscesses in the non-granulomatous peripheral inflammatory infiltrate was observed in 8 (10.5%) cases.

Table 1 Quantity of each cell type present in the infiltrate and its association with granuloma differentiation

Cell type	Quantity	Predominant differentiation of mononuclear phagocytes in granuloma	
		Macrophages ($n = 21$)	Epithelioid cells ($n = 59$)
NIG ($n = 80$)	Discrete to moderate	10	28
	Elevated	11	31
PAG ($n = 80$)	Ausent	4	4
	Discrete to moderate	11	35
	Elevated	6	20
PPL ($n = 80$)	Ausent	3	10
	Discrete to moderate	10	30
	Elevated	8	19
LPL ($n = 80$)	Ausent	4	20
	Discrete to moderate	12	22
	Elevated	5	17
NPL ($n = 80$)	Ausent	7	36
	Discrete to moderate	5	10
	Elevated	9	13
MPL ($n = 80$)	Ausent	18	40
	Discret to moderate	3	19
	Elevated	–	–

NIG Neutrophils inside granuloma, *PAG* plasma cells around granuloma, *PPL* plasma cells at the periphery of the lesion, *LPL* lymphocytes at the periphery of the lesion, *NPL* neutrophils at the periphery of the lesion, *MPL* macrophages at the periphery of the lesion

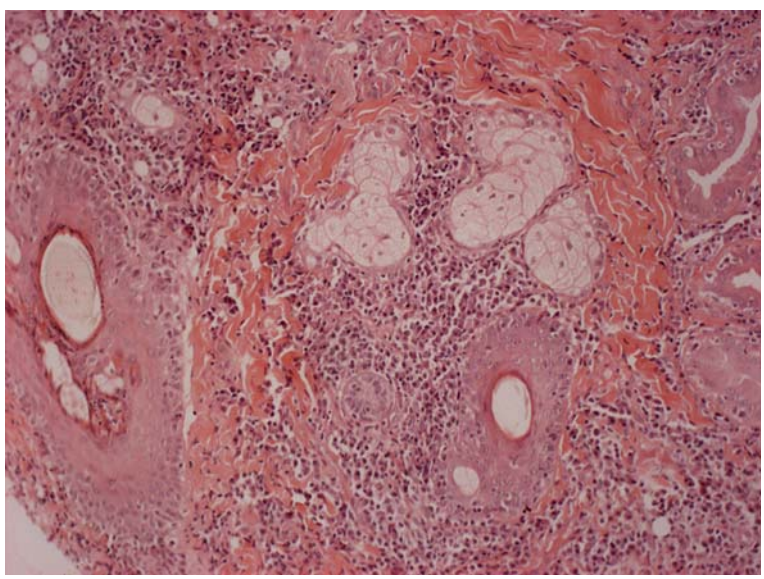

Fig. 4 Perifollicular plasmacellular infiltrate. HE stain. 20×

The epidermis exhibited ulceration in 23.7% of the cases and hyperplasia in 10.5%, and the simultaneous presence of both alterations was observed in 2 cases. Edema was present in 21.0% of the cases and intraepithelial microabscesses in 4 (5.3%).

Non-suppurative Granulomatous Inflammation

Microscopically, the lesion was characterized by poorly organized, diffuse granulomas with a predominance of epithelioid cells. These granulomas were

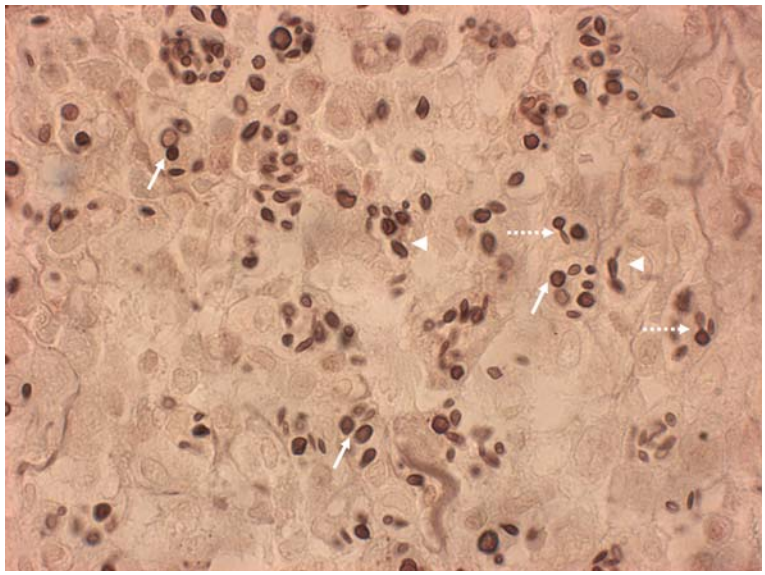

Fig. 5 Round (*Arrows*) and cigar-shaped yeast cells (*Arrows heads*) presenting single buds with a narrow base (*Dashed arrows*). Grocott. 100×

surrounded by a small number of plasma cells. The non-granulomatous peripheral inflammatory infiltrate showed a perivascular, perifollicular and interstitial distribution and contained large quantities of plasma cells and moderate quantities of lymphocytes and neutrophils.

Nonspecific Inflammation

Nonspecific inflammation was observed in five cases. Plasma cells were detected in all cases, neutrophils in three, lymphocytes in two, and macrophages and eosinophils in one. Two cases presented an essentially plasma-cell infiltrate. The distribution of the infiltrate was perivascular, perifollicular and interstitial in four cases, with one of these cases also showing lichenoid infiltration. A diffuse pattern was observed in one case. Dermal edema was noted in three cases.

Presence of Yeast Cells Compatible with *S. schenckii*

Yeast cells were only detected in lesions with suppurative granulomas. Silver and PAS staining revealed yeast cells in 36 (41.9%) and 17 (19.8%) cases, respectively, demonstrating a higher sensitivity of the former technique. In addition, none of the cases testing negative by silver staining was positive by PAS. Twenty-one (58.3%) of the 36 positive cases presented a maximum of five fungal elements, and a large number of yeast cells in the histological sections (more than 25) was only observed in 8 (22.2%) of these positive cases (Fig. 5).

The yeast cells were mainly round or oval, with cigar-shaped cells being detected only in lesions rich in fungus. Budding yeast cells were observed in 17 (47.2%) of the positive cases (Fig. 5). Most of the yeast cells detected were located inside the granulomas, especially in the suppurative areas.

Yeast cells were detected in 56.4% and 37.8% of the cases that presented an elevated ($n = 39$) and discrete to moderate ($n = 37$) number of neutrophils inside the granuloma, respectively. In addition, a large number of neutrophils inside the granuloma and the presence of poorly organized granulomas were observed in seven of the eight cases in which large numbers of fungi were detected. No asteroid bodies were detected in the cases studied by any of the staining techniques used.

Discussion

The canine sporotrichosis lesions analyzed here were microscopically characterized by suppurative granulomatous processes similar to descriptions in humans [17]. The absence of histopathological alterations in four cases probably reflects a nonrepresentative

sample of the lesion or wear and tear of the paraffin block, since all cases included in the study presented clinically active lesions.

The granulomas observed generally contained neutrophilic foci and were surrounded by lymphocytes and plasma cells as described by Lurie [17] for sporotrichotic granuloma. Sporotrichotic granuloma was a frequent finding in the present study and in previous investigations [17, 22, 25], but is also observed in other lesions of fungal etiology [14].

The presence of giant cells, which is a common finding in sporotrichotic granulomas in humans [13, 17, 19, 22, 31], was rare in this study. Similar findings have been reported in one human case [24] and in experimentally infected mice [20].

Pseudoepitheliomatous hyperplasia, a frequent finding in human disease [13, 17, 22], was not observed. Intraepithelial microabscesses, also common according to several investigators [13, 17, 22], were detected in only four (4.65%) cases. These abscesses are not specific for sporotrichosis and can be found in lesions caused by other fungi [14].

The frequent presence of a peripheral inflammatory infiltrate around the lesion observed in the dogs studied here has also been reported for humans [13, 17, 22, 30, 31]. The infiltrate mainly consisted of plasma cells and showed a perivascular, perifollicular and interstitial distribution. The lichenoid infiltrate detected was always accompanied by perivascular, perifollicular and interstitial infiltration. Although a lichenoid infiltrate is generally found in noninfectious inflammatory skin diseases [37], it was detected in only a few cases and was considered to be an incidental morphological pattern.

According to experimental studies conducted on humans and mice, the initial course of sporotrichosis lesions is characterized by the formation of abscesses and an elevated number of fungi. Next, the number of fungi and neutrophils seems to decline with the arrival and activation of mononuclear phagocytes which develop into epithelioid cells, accompanied by an increase in lymphocytes and plasma cells [18, 19, 38]. Similar observations regarding granuloma formation have been made in experimental studies using Bacillus Calmette-Guérin (BCG), which elicits the morphological transformation of mononuclear phagocytes from immature macrophages during early stages into mature epithelioid cells found in well-organized granulomas [21, 39]. In the present study, plasma cells surrounding the granuloma were more frequent in granulomas that mainly consisted of epithelioid cells compared to those containing macrophages, a finding that might be related to the duration of the disease as reported by other investigators [21, 39]. In addition, the difference in the number of neutrophils between granulomas consisting of macrophages and those consisting of epithelioid cells was significant, suggesting that in canine sporotrichosis less-differentiated granulomas mainly consisting of macrophages are found in the more acute phases and are later replaced with more mature granulomas consisting of epithelioid cells, a fact reported in experimental studies on other pathogens [21].

In the present investigation, analysis of four sections per case yielded a higher sensitivity (41.9%) than those observed in most studies reporting detection rates of less than 30% [11, 16, 22, 23, 40]. Grocott's silver stain showed a higher sensitivity than PAS. Similar results have been reported by Marques et al. [40] for humans. In view of the scarcity of yeast cells, analysis of serial sections obtained from the same biopsies may markedly increase the chance of detection of the agent in suppurative granulomatous inflammation. In fact, other investigators reported satisfactory sensitivity of histological methods for the detection of the agent when multiple sections were analyzed [22].

The presence of large numbers of the agent in histological sections, as observed in some dogs of this study, has also been described in humans [31]. Lesions rich in fungi, which are generally observed in more severe forms of the disease, seem to be related to several factors including the presence of concomitant diseases, malnutrition and the use of immunosuppressive drugs such as corticosteroids [11, 41, 42]. According to some studies, the number of fungal elements is initially high and tends to decrease with the formation and maturation of granulomas [18, 19, 38]. In the present investigation, most lesions containing an elevated number of fungi accompanied granulomas that were poorly organized and rich in neutrophils. According to experimental findings, this is consistent with early lesions, suggesting that an early examination may increase the possibility of detection of the agent [19, 20, 38]. In agreement with the present study, Moraes and Miranda [22] also obtained higher positivity in cases of granulomas containing an elevated number of neutrophils.

The presence of asteroid bodies of *S. schenckii* has been considered to be a frequent finding in some studies [13, 17, 22, 30], whereas this structure was not detected in another investigation [31] nor in the present study. According to Pinkus and Grekin [30], it is unlikely that this structure is not recognized upon lesion analysis because of its large size.

According to other investigators [22] and in agreement with the present study, *S. schenckii* yeast cells are generally located in the center of suppurative granulomas inside microabscesses, a fact that seems to contribute to the detection of the agent in suspected sporotrichosis lesions.

As previously described for humans [17, 22], the present study demonstrated that skin lesions exhibiting suppurative granulomas surrounded by lymphocytes and plasma cells and associated with a predominantly plasma-cell peripheral infiltrate are highly suggestive of canine sporotrichosis. However, these findings are not specific since they have also been described in skin lesions of American cutaneous leishmaniasis and other fungal diseases affecting both dogs and humans [11, 16, 23, 34, 43].

In Rio de Janeiro, the distinction between American cutaneous leishmaniasis and sporotrichosis is imperative due to overlapping endemic areas and clinical-epidemiological and laboratory similarities of these diseases [11, 16, 23]. In histopathological samples, in addition to the possible similarity of microscopic alterations, the differentiation between *S. schenckii* yeast cells and *Leishmania* sp. amastigotes might be difficult when HE staining is used because of the similar size and shape of the two forms [11, 16], a fact requiring the use of specific staining techniques.

In conclusion, in the case of dogs with skin lesions whose histopathological presentation is consistent with that of sporotrichosis, serial sections of the lesion stained by techniques for the detection of fungi are recommended, with Grocott's silver staining being the most effective method. However, due to the generally small number of yeast cells in the lesions, the diagnostic hypothesis of sporotrichosis should not be ruled out even when this test is negative, especially in areas where the epidemic occurs. In these cases, correlation with epidemiological data is particularly useful.

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References

1. Schell W. Agents of chromoblastomycosis and sporotrichosis. In: Allejo L, Hay RJ, editors. Topley & Wilson's—microbiology and microbial infectious. London: Arnold; 1998. p. 315–36.
2. Dunstan RW, Langham RF, Reinman KA, Wakenell OS. Feline sporotrichosis: a report of five cases with transmission to humans. J Am Acad Dermatol. 1986;15:37–45. doi:10.1016/S0190-9622(86)70139-4.
3. Kauffman CA. Sporotrichosis. Clin Infect Dis. 1999;29:231–6. doi:10.1086/520190.
4. Barros MBL, Schubach AO, Galhardo MCG, Schubach TMP, Reis RS, Conceição MJ, et al. Sporotrichosis with widespread cutaneous lesions: report of 24 cases related to transmission by domestic cats in Rio de Janeiro, Brazil. Int J Dermatol. 2003;42:677–81. doi:10.1046/j.1365-4362.2003.01813.x.
5. Barros MBL, Schubach AO, do Valle ACF, Galhardo MCG, Conceição-Silva F, Schubach TMP, et al. Cat-transmitted sporotrichosis epidemic in Rio de Janeiro, Brazil: description of a series of cases. Clin Infect Dis. 2004;38:529–35. doi:10.1086/381200.
6. Schubach TMP, Schubach AO, Okamoto T, Barros MB, Figueiredo FB, Cuzzi T, et al. Evaluation of an epidemic of sporotrichosis in cats: 347 cases (1998–2001). J Am Vet Med Assoc. 2004;224:1623–9. doi:10.2460/javma.2004.224.1623.
7. Freitas D, Moreno G, Saliba A, Bottino J, Mós E. Esporotricose em cães e gatos. Rev Fac Med Vet Univ Sao Paulo. 1965;7:381–7.
8. Kier A, Mann P, Wagner J. Disseminated sporotrichosis in a cat. J Am Vet Med Assoc. 1979;175:202–4.
9. Gonzalez-Cabo J, Guilamon MH, Cequiel ML, Ciercoles JGJ. Feline sporotrichosis: a case report. Mycopathologia. 1989;108:149–54. doi:10.1007/BF00436219.
10. Barros MBL, Schubach TMP, Galhardo MCG, Schubach AO, Fialho-Monteiro PC, Reis RS, et al. Sporotrichosis: an emergent zoonosis in Rio de Janeiro. Mem Inst Oswaldo Cruz. 2001;96:777–9.
11. Schubach TMP, Schubach AO, Okamoto T, Barros MBL, Figueiredo FB, Cuzzi T, et al. Canine sporotrichosis in Rio de Janeiro, Brazil: clinical presentation, laboratory diagnosis and therapeutic response in 44 cases (1998–2003). Med Mycol. 2006;44:87–92. doi:10.1080/13693780500148186.
12. Kwon-Chung K, Bennet J. Sporotrichosis. In: Kwon-Chung K, Bennet J, editors. Medical mycology. Philadelphia: Lea & Febiger; 1992. p. 707.

13. Moore M, Ackerman LV. Sporotrichosis with radiate formation in tissue: report of a case. Arch Derm Syphilol. 1946;53:253–64.
14. Schwarz J. The diagnosis of deep mycoses by morphologic methods. Hum Pathol. 1982;13:519–33.
15. Rodríguez G, Sarmiento L. The asteroid bodies of sporotrichosis. Am J Dermatopathol. 1998;20:246–9. doi:10.1097/00000372-199806000-00004.
16. Barros MBL, Schubach AO, do Valle ACF, Galhardo MCG, Schubach TMP, Conceição-Silva F, et al. Positive Montenegro skin test among patients with sporotrichosis in Rio de Janeiro. Acta Trop. 2005;93:41–7. doi:10.1016/j.actatropica.2004.09.004.
17. Lurie HI. Histopathology of sporotrichosis—notes of the nature of the asteroid body. Arch Path. 1963;75:421–37.
18. Miyaji M, Nishimura K. Defensive role of granuloma against *Sporothrix schenckii* infection. Mycopathologia. 1982;80:117–24.
19. Mohri S. Study in sporotrichosis—III histological and immunohistochemical study in experimental cutaneous sporotrichosis in man. Yokohama Med Bull. 1987;38(1–2):37–48.
20. Hiruma M, Yamaji K, Shimizu H, Ohata H, Kukita A. Ultrastructural study of tissue reaction of mice against *Sporothrix schenckii* infection. Arch Dermatol Res. 1988;280:S94–100.
21. Adams DO. The structure of mononuclear phagocytes differentiation in vivo—I. Sequential fine histological studies of the effect of Bacillus Calmette-Guerin (BCG). Am J Pathol. 1974;76(1):17–39.
22. Moraes MAP, Miranda EV. On the presence of radiate (asteroid) formations in sporotrichosis. Rev Inst Med Trop Sao Paulo. 1964;6(1):5–11.
23. Santos IB, Schubach TMP, Leme LRP, Okamoto T, Figueiredo FB, Pereira SA, et al. Sporotrichosis—the main differential diagnosis with tegumentary leishmaniosis in dogs from Rio de Janeiro, Brazil. Vet Parasitol. 2007;143:1–6. doi:10.1016/j.vetpar.2006.08.002.
24. Moskowitz LB, Ganjei P, Ziegels-Weissman J, Clearly TJ, Penneys NS, Nadji M. Immunohistological identification of fungi in systemic and cutaneous mycoses. Arch Pathol Lab Med. 1986;110:433–6.
25. Marques MEA, Coelho KIR, Sotto MN, Bacchi C. Comparison between histochemical and immunohistochemical methods for diagnosis of sporotrichosis. J Clin Pathol. 1992;45:1089–93. doi:10.1136/jcp.45.12.1089.
26. Reed JA, Hemann BA, Alexander JL, Brigati DJ. Immunomycology: rapid and specific immunohistochemical identification of fungi in formalin-fixed, paraffin-embedded material. J Histochem Cytochem. 1993;41(8):1217–21.
27. Rodriguez G, Sarmiento L. The asteroid bodies of sporotrichosis. Am J Dermatopathol. 1998;20:246–9. doi:10.1097/00000372-199806000-00004.
28. Hu S, Chung WH, Hung SI, Ho HC, Wang ZW, Chen CH, et al. Detection of *Sporothrix schenckii* in clinical samples by a nested PCR assay. J Clin Microbiol. 2003;41(4):1414–8. doi:10.1128/JCM.41.4.1414-1418.2003.
29. Kanbe T, Natsume L, Goto I, Kawasaki M, Mochisuki T, Ishizaki H, et al. Rapid and specific identification of *Sporothrix schenckii* by PCR targeting the DNA topoisomerase II gene. J Dermatol Sci. 2005;38:99–106. doi:10.1016/j.jdermsci.2004.12.024.
30. Pinkus H, Grekin JN. Sporotrichosis with asteroid tissue forms—report of a case. Arch Derm Syphilol. 1950;61:813–9.
31. Gadelha AR, Talhari S. Demonstração do *Sporothrix schenckii* em cortes histológicos. Med Cutan Ibero Lat Am. 1982;10(6):355–60.
32. Narisawa Y, Kojima T, Iriki A, Masaki J, Kohda H. Tissue changes in cryptococcosis: histologic alteration from gelatinous to suppurative granulomatous tissue response with asteroid body. Mycopathologia. 1989;106:113–9. doi:10.1007/BF00437090.
33. Moore M. Radiate formation on pathogenic fungi in human tissue. Arch Pathol. 1946;42:113–53.
34. Koehne G, Powell HS, Hail RI. Sporotrichosis in a dog. J Am Vet Med Assoc. 1971;159(7):892–4.
35. Sykes JE, Torres SM, Armstrong PJ, Lindeman CJ. Itraconazole for treatment of sporotrichosis in a dog residing on a Christmas tree farm. J Am Vet Med Assoc. 2001;218(9):1440–3. doi:10.2460/javma.2001.218.1440.
36. Ackerman AB, Chongchitnant N, Sanchez J, Guo Y, Bennin B, Reichel M, et al. Histologic diagnosis of inflammatory skin diseases: an algorithmic method based on pattern analysis. 2nd ed. Baltimore: Williams & Wilkins; 1997.
37. Gross TL, Ihrke PJ, Walder EJ. Lichenoid (interface) diseases of the dermis. In: Gross TL, editor. Veterinary dermatopathology: a macroscopic and microscopic evaluation of canine and feline skin disease. St. Louis: Mosby-Year Book; 1992. p. 141–62.
38. Peng-Cheng L, Yoshiike T, Yaguchi H, Ogawa H. Histopathological studies of *Sporothrix schenckii*-inoculated mice—possible functions of polymorphonuclear leukocytes in normal and immunocompromised (congenitally athymic nude) mice. Mycopathologia. 1993;122:89–93. doi:10.1007/BF01103604.
39. Adams DO. The granulomatous inflammatory response—a review. Am J Pathol. 1976;84(1):164–91.
40. Marques MEA, Coelho KIR, Sotto MN, Bacchi CE. Comparison between histochemical and immunohistochemical methods for diagnosis of sporotrichosis. J Clin Pathol. 1992;45:1089–93. doi:10.1136/jcp.45.12.1089.
41. Bernstein JA, Cook HE, Gill AF, Ryan KA, Sirninger J. Cytologic diagnosis of generalized cutaneous sporotrichosis in a hunting hound. Vet Clin Pathol. 2007;36(1):94–6. doi:10.1111/j.1939-165X.2007.tb00189.x.
42. Carfachia C, Sasanelli MT, Lia RP, Caprariis D, Guillot J, Otranto D. Lymphocutaneous and nasal sporotrichosis in a dog from Southern Italy: case report. Mycopathologia. 2007;163:75–9. doi:10.1007/s11046-006-0086-x.
43. Welsh RD. Sporotrichosis. J Am Vet Med Assoc. 2003;223(8):1123–6. doi:10.2460/javma.2003.223.1123.